

*Full Length Research Paper*

# Seasonal distribution of soil fungi and chemical properties of montane wet temperate forest types of Tamil Nadu

K. Saravanakumar and V. Kaviyarasan\*

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai-600 025, Tamil Nadu, India.

Accepted 20 April, 2010

Forty eight soil samples were collected from Wet evergreen forest of Tamil Nadu, Southern India; the fungi from these soil samples were isolated in (June – July) South West monsoon and (November – December) North East resting monsoon both seasons. About different species belongs to various groups viz; Ascomycotina, Zygomycotina and Deuteromycotina were identified with the help of relevant literatures. A total of 76 taxa belonging to 25 genera were isolated, these include one species of Acomycetes, one species of Coelomycetes five species of Zygomycetes and remaining species were Deuteromycetes. Twenty one species of *Penicillium* and 14 species of *Aspergillus* were also recorded from both seasons. None of the basidiomycetes could be isolated from these soils in spite of our best efforts. The diversity indices of forest soil fungi over the two seasons were 2.953, 2.699 (Shannon-Weinner), 0.9033, 0.8491 (Simpson index) and 0.2219, 0.3485 (Fishers's alpha), respectively. The soil nutrients were also analyzed for montane wet temperate forest. The macro nutrients such as N, P, K content were rich in after the raining season and organic content of natural soil was also increased.

**Key words:** Soil fungi, diversity, forest soil, seasonal distribution, soil nutrients.

## INTRODUCTION

The relationship between biodiversity of soil fungi and ecosystem function is an issue of paramount importance, particularly in the face of global climate change and human alteration of ecosystem processes. Fungi are an important component of the soil micro biota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth and Bisby, 1995). The saprobic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in the decomposition of plant structural polymers, such as, cellulose, hemicelluloses and lignin, thus contributing to the maintenance of global carbon cycle. Many of the papers on the distribution of fungi in soil have dealt with species in agricultural soils and less is known about the occurrence of fungi under

natural soil conditions (Ling-Young, 1930). The question of an ecological distribution of fungi in such soils has received little attention. Ling-Young (1930) pointed out that in order to obtain a fair idea of an endemic micro flora; soils should be examined from such localities as forests, peat bogs and mountains which remain untouched by man. From the numerous studies on soil fungi there has gradually grown up the concept of a fungal flora of the soil, fungal flora may vary depends on its native soils (Shi et al., 2002; Gleason et al., 2004; Burges, 1939). Some fungi are widely distributed in soil and others are limited to certain habitats.

The distribution of these organisms is influenced by the abundance and nature of the organic content of the soil, as well as by other soil and climatic conditions, surface vegetation and soil texture (Waksman, 1944; Marschner et al., 2003). While the general nature of the soil micro flora has become recognized, details, particularly the ecological variation of its members, are not always clear. The present study was undertaken to investigate soil

\*Corresponding author. E-mail: [manikavi53@gmail.com](mailto:manikavi53@gmail.com). Tel. +91-9444255225. Fax: +91-44-2352494.

**Table 1.** Nutrient analysis table of forest soils.

Macro and micro nutrients	Season I	Season II
Soil moisture	14.2	1805
Soil pH	4.506	4.73
Electrical conductivity (Dsm-1)	0.0680	0.650
Nitrogen Kg/ac	131.474	169.6
Organic carbon %	1.54	1.855
P <sub>2</sub> O <sub>5</sub> Kg/ac	10.946	13.13
K <sub>2</sub> O Kg/ac	37.843	49.4
Iron (ppm conc)	16.79	18.957
Copper (ppm conc)	0.690	0.66
Manganese (ppm conc)	5.561	6.01
Zinc (ppm conc)	0.528	0.510

nutrients and soil mycoflora of natural soils of montane wet temperate forest Tamil Nadu.

## METHODOLOGY

### Study site and location

Tropical montane forests are situated in the higher mountain tracts of the Southern Western Ghats, at an altitude above 1500 m, and above these MSL interspersed with rolling grasslands. The forest area contains 21.45 sq.km of Reserved Forests and 4000 Ha. of Reserved Land, located in Dindigul revenue district and lies within 10°6' and 10°21' North latitudes and 77°16' and 77°42' East longitudes and is surrounded by Kerala State in West, Indra Gandhi Wild life Sanctuary, Pollachi in Northwest, Dindigul forest division on the North and East and Theni revenue district in South. The altitude varies from 300 to 2654 m. The annual average rainfall 165 cm and the minimum temperature of Kodaikanal vary between 8 to 13°C and the maximum temperature varies between 11.3 to 19.8°C.

The Shola forests (Tropical montane forest) exhibit high biodiversity and these forests are rare in the world. The plant families *Lauraceae* and *Rubiaceae* are well represented in the sholas. *Acanthaceae* members dominated among the shrubs. Many species of *Strobilanthes* and *Andrographis* (both belonging to *Acanthaceae*) are endemic to this region. In the Western Ghats, members of the flora families like *Ranunculaceae*, *Geraniaceae*, and *Saxifragaceae* are seen only in such regimes.

### Methods for collection of soil samples

The soil samples were collected from June - July (South-west monsoon (raining (II) season) and the end of November-December (North-east resting monsoon) after summer rainfall period (I season) during 2007 - 2008, the flora of forest areas remain covered for a long time by plant debris. In the present case, each sample was collected from Shola forests of Tamil Nadu, such as Governor Shola (6 samples) - Ooty, Long wood Shola (6 sample) - Kothagiri, Vandi Shola (6 samples) - Ooty, Glenmorgan (6 samples) - Ooty of Nilgiris, Mathikettan Shola (6 samples), Gugal Shola (6 samples), Pampar Shola (6 samples) and Gundar Shola

(6 samples) of Kodaikanal.

The method used for taking soil samples was a slight modification as that used by Goddard (1913). It was slightly modified by Saksena and Mehrotra (1952) for their studies. The soil sample was collected up to 30 cm deep from the surface soil (removal of surface organic matter), each sample contains about 100 g of soil and was kept in sterile polythene bags and brought to the laboratory. All random soil samples of each site were put together to make a single sample for each forest.

### Determination of physicochemical properties of soil samples

The pH values, electrical conductivity, soil moisture, organic carbon, nitrogen, phosphorous, potassium, iron, manganese, copper and zinc were analyzed (Table 1). The macro nutrients such as Nitrogen (Alkali permanganate method), phosphorous (Olsen method), potassium (neutral normal ammonium acetate method), organic carbon (Walkley and Block method) and micro nutrients such as copper, iron, manganese and zinc were analyzed by DTPA extract method using atomic absorption spectrophotometer.

### Isolation of soil mycoflora

The soil micro fungi were enumerated by two methods, namely, Soil dilution, (Waksman, 1927), and Soil plate method (Warcup, 1950) on different media such as potato dextrose agar, Czapek's Dox and Rose Bengal agar at pH 6.5. All the Petri dishes were incubated at room temperature  $27 \pm 3^\circ\text{C}$  for a period of 4 - 7 days and then examined.

The first set of observations were made at the end of two days to make sure that the fast growing flocculent types such as *Rhizopus*, *Mucor* and *Trichoderma*, etc., has grown excessively to interfere with observations of other species. Second observation was made when these had come to an advanced stage to enable identification. Finally, the slow growing organisms has to be sub-cultured in different media for the purpose of further growth to save them from being overrun by the more aggressive types. The number of colonies per plate in 1 g of soil was calculated.

### Identification

Identification of the organisms was made by microscopic analysis using taxonomic guides, standard procedures and relevant literature (Kenneth and Dorothy, 1965; Kenneth and Dorothy, 1968; Domsch et al., 1980 and Ellis, 1971). While presenting the data two terms, viz; periodicity of occurrence and 'percent contribution and statistical analysis were used. The percent contribution of each isolate was calculated by using the following formula:

$$\frac{\text{Total no. of CFU of an individual species}}{\text{Total no. of CFU of all species}} \times 100$$

The periodicity of occurrence denotes the number of samplings in which a fungus was present as against the total number of samplings. The periodicity of occurrence was calculated for fungi are arbitrarily classified as follows:

Common - recorded in 10 - 15 samplings  
 Frequent - recorded in 7 - 9 samplings  
 Occasional - recorded in 4 - 6 samplings  
 Rare - recorded in 1 - 3 samplings

The following indices were analyzed;

#### Shannon–Wiener index: 2

$$H' = -\sum_{i=1}^S p_i \log_2 p_i$$

Where  $S$  is the number of OTUs (Operational Taxonomic Units) and  $p_i$  is the proportion of total samples belonging to the  $i$ th OTU.  $H'$  varies between 0 and  $\log_2 S$  is the information content of the relevant sample (units, bits per OTU).  $H'$  close to 0 indicates low diversity; whereas a value close to  $\log_2 S$  indicates high diversity.

#### Simpson's index (modified by Pielou)

$$1 - D = 1 - \sum_{i=1}^S \frac{n_i(n_i-1)}{[N(N-1)]}$$

Where  $n_i$  is the number of individuals in the  $i$ th OTU,  $S$  is the total number of OTUs and  $N$  is the total number of individuals. The diversity is minimum when only one OTU exists, that is if  $n_i = N$  for some  $i$  and  $n_i = 0$  otherwise,  $1 - D = 0$ . It is a maximum when all species are represented equally (each  $n_i = N/S$ ). Then  $1 - D = (1 - 1/S)$  approximately for large values of  $N$ .

#### Fisher's index ('alpha diversity')

$$S = \alpha \ln(1 + N/\alpha),$$

Where  $S$  is the number of OTUs in the sample,  $N$  is the number of individuals in the sample and  $\alpha$  is the Fisher's index of diversity. The assumption here is that the number of OTUs increases logarithmically with the number of individuals. If so,  $\alpha$  is a measure of the rate of increase of the number of OTUs with respect to increasing (logarithmic) population size when the size is large.

#### Evenness index (1)

$$E = H' / \ln(S),$$

Where  $H'$  is the Shannon–Wiener index of diversity

## RESULTS AND DISCUSSIONS

### Soil nutrient analysis

The soil pH, organic content and water are the main factors affecting the fungal population and diversity (Yu et al., 2007; Dong et al., 2004; Song et al., 2004; Zhang et al., 2001; Ju et al., 2008). Organic carbon largely controls microbial growth in the natural soil. It is a key factor governing Nitrogen, Phosphorous and Sulphur cycles. Physical and chemical parameters of forest soil such as pH, electrical conductivity, moisture content, macro nutrients (Nitrogen, organic Carbon, Phosphorous, Potassium) and micro nutrients (Fe, Mn, Cu, and Zn) were carried out in detail and were compared with both season of Montane wet temperate forest which are represented in Table 1. Analysis of forest soils revealed very high moisture and organic contents. In all the soils investigated, the pH and electrical conductivity were almost same in both seasons, whereas, the macro nutrient

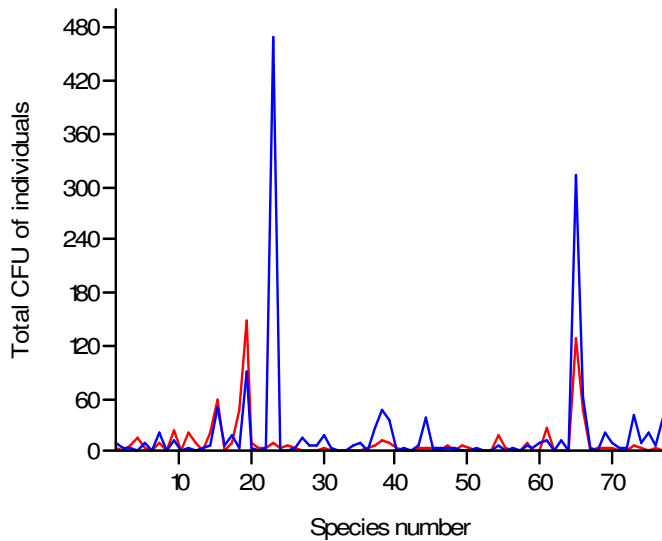
like nitrogen was the most prominent soil mineral nutrients in Montane wet temperate forest. The Nitrogen, Phosphorous and Potassium content were very rich in the second season viz; 169.6, 13.13 and 49.4%, respectively. But the organic Carbon was slightly increased than the first season.

However, the micro nutrients such as, Iron and Manganese were also significantly higher but in the case of Copper and Zinc was decreased in the second season of Montane wet temperate forest. (Table 1) The organic carbon, nitrogen, phosphorous, potassium are important for fungi. In the absence of any one of these the growth and sporulation of moulds as well as other micro organisms are hampered a lot. Magnesium, manganese and iron though needed in very small quantities, are also essential (Saksena, 1955). The availability of micro nutrients such as Fe, Mn, Cu and Zn was relatively 1 – 25 ppm concentration (Alexander, 1986).

Forty two soil samples were collected for microbial enumeration from Shola forests of Tamil Nadu, such as Governor Shola (6 samples) – Ooty, Long wood Shola (6 samples) – Kothagiri, Vandi Shola (6 samples) – Ooty, Glenmorgan (6 samples) – Ooty of Nilgiris District. Mathikettan Shola (6 samples), Gugal Shola (6 samples), Pampar Shola (6 samples), and Gundar Shola (6 samples) of Kodaikanal – Dindigul. Out of 48 samples 55 species belonged to 21 genera were recorded. Among these two of *Ascomycotina*, nine species of *Zygomycotina* and others belonged to *Deuteromycotina*. *Aspergillus niger* was the most dominant species with 20.76%, and *Penicillium* sp MDU (18.07 %) was occurred second in the order of dominance, the *Aspergillus flavus* and *Trichoderma aureoviride* contributed equally (6.49%). These were followed by *Penicillium simplicissimum*, *Rhizopus stolonifer*, *Syncephalastrum racemosum*, *Aspergillus fisheri* and *Cunninghamella echinulata* to the total biodiversity ( $H' = 2.953$ ).

However, sixty seven species belongs to 23 genera were recorded from second season among them six species of *Zygomycotina*, two of *Ascomycotina*, four species of *Coelomycetes* and remaining fifty five species are *Deuteromycetes*. *Penicillium* spp., are the predominant genera (19) and followed by *Aspergillus* with twelve species. Similarly, Asan (1997) studied the flora of *Penicillium* and *Aspergillus* in different habitat soils in Edrine. He found twenty three species and 2 varieties belonging to *Aspergillus* and sixteen species belonging to *Penicillium*. Earlier reports also indicate that *Aspergillus* and *Penicillium* were dominant in forest soils (Galloway, 1936) and Moubasher and El-Dohlob (1970). In our case, the pattern of distribution were common forms, though not rare forms, repeats it over the two seasons. The genera of *Curvularia* (7 species), *Trichoderma* (5 species), *Fusarium* (3 species), *Cladosporium* (2 species), *Paecilomyces* (2 species) contributed more than one species and other genera were represented as single.

*Aspergillus terreus* occurred in highest contribution



**Figure 1.** Distribution of fungal flora of montane wet temperate forest. Representation of total CFU of an individual species in the South West monsoon (II season) and North East resting monsoon (I season), the number denotes that particular species listed in the table.

(30.75%) and *Penicillium* spp. MDU was the second dominant flora with 20.52% and followed by *Aspergillus niger* (5.84%), *T. areoviride* (4.06%), *F. oxysporum* (2.95%) and *A. flavus* (3.21%). *P. veruculosum*, *Penicillium* spp. (6), *A. amstelodami*, *Curvularia oryzae* and *Nigrospora sphaerica* were contributed as very low mentioned in Table 3 and Figure 1). Most of the Zygomycotina members were recorded from both seasons of montane wet temperate forest. The genus of *Penicillium* was most dominant and widespread in both seasons. Hasenekoglu (1985) performed quantitative analysis of the micro flora of forest, grass land and field soils in vicinity of Sarikamis. He reported that the genus *Penicillium* was the most common in terms of species and intensity in his research. Chaudhary and Sachar (1934), Saksena (1955), Miller et al. (1957) and Saksena and Sarbhoy (1964) studied seasonal variation in soil myco-flora and fungal population, which drastically differ from season to season in a particular soil. Trenser et al. (1954), Miller et al. (1957), Mishra (1966), Rama rao (1969), Persiani et al. (1998) and many others also observed seasonal variations in forest soil mycoflora.

### Periodicity of occurrence

As it has already been stated in the 'methodology', the fungi have been classified into four categories depending on the number of samplings in which a particular species was recorded as against the total number of samplings. Thus, among the total species recorded, fourteen species

**Table 2.** Diversity indices of two seasons of Montane wet temperate forest.

Diversity indices	Montane wet temperate forest	
	Season - I	Season -II
Taxa_S	55	67
Dominance_D	0.09671	0.1509
Shannon_H	2.953	2.699
Simpson_1-D	0.9033	0.8491
Evenness_e^H/S	0.2219	0.3485
Fisher_alpha	13.9	14.37

occurred most common; *A. niger*, *A. japonicus* and *A. flavus* were most commonly encountered (Table 3), even among these, *A. niger*, *A. flavus* and *A. terreus* were recorded in all the samplings while the other eleven were recorded 10 - 14 samplings. Seven species were recorded frequent. Among others, twenty species were occasional and remaining thirty one species were rare in their occurrence. *Rhizopus stolonifer* was recorded in highest number of samplings when compared with other species of Zygomycotina.

The highest total number of taxa\_S was recorded in the second season of Montane wet forest when compared to first season. However, the Fisher alpha test was significantly different in the both seasons (Table 2). All our estimates of Simpson index are close to 1, meaning that the probability is very low. Further, a number of indices of fungal diversity – number of genera, Shannon – Weiner index and Simpson index were almost similar to both seasons. This may be a reflection of the fact that we have monitored only cultivable living soil fungi and not from fungi associated with plants and trees (Nilima satish et al., 2007). The species Evenness\_e^H/S was highest in the second season of montane wet forest, in the case of total taxa\_S was also higher. The total number of fungal genera increases with the area monitored. Thomas and Shattock (1986) also found that the logarithmic and log normal distributions were best studied for a description of the abundance of 33 genera of filamentous fungi (Krebs, 1989).

Reported values of soil fungal diversity and population density are often a reflection of the methods used to recover the fungi, with optimal sampling methods differ from organism to organism (Brock, 1987; Schlegel and Jannasch, 1972). Identification is complicated by the fact that fungal life cycles in the soil and in the laboratory can be quite different. Therefore, instead of attempting species identification, many researchers classify individuals at the generic level (Donnel et al., 1994). Fungi are so nutritionally diverse that there is no single medium that can be used to isolate all of them. The technique of direct isolation from particles of soil would have yielded more counts, but the fast growing fungi would still be favored Galloway (1936).

**Table 3.** Mycoflora of montane wet temperate forest.

Name of fungal species	Season I		Season II		Periodicity of occurrence
	Total CFU × 10 <sup>3</sup>	Percent contribution	Total CFU × 10 <sup>3</sup>	Percent Contribution	
<b>Ascomycotina</b>					
<i>Chaetomium globosum</i>	3	0.42	9	0.6	O
<i>C. cochliodes</i>	--	--	2	0.13	R
<b>Zygomycotina</b>					
<i>Absidia cylindrospora</i>	6	0.84	3	0.19	O
<i>Cunninghamella echinulata</i>	14	1.98	--	--	F
<i>C. elegans</i>	2	0.28	8	0.52	O
<i>Mortierella elongate</i>	1	0.14	1	0.06	R
<i>Mucor circinelloides</i>	8	1.12	19	1.25	F
<i>M. racemosus</i>	1	0.14	--	--	R
<i>Rhizopus stolonifer</i>	24	3.38	11	--	C
<i>Rhizomucor pusillus</i>	--	--	1	0.06	R
<i>Syncephalastrum racemosum</i>	19	2.68	2	0.13	C
<b>Deuteromycotina</b>					
<i>Aspergillus amstelodami</i>	8	1.12	1	0.06	O
<i>A. hevalieri</i>	1	0.14	2	0.13	R
<i>A. fisherii</i>	20	2.82	7	0.46	O
<i>A. flavus</i>	58	8.19	49	3.21	C
<i>A. flaviceps</i>	--	--	5	0.32	O
<i>A. fumigatus</i>	8	1.12	16	1.05	C
<i>A. japonicus</i>	46	6.49	2	0.13	C
<i>A. niger</i>	147	20.76	89	5.84	C
<i>A. nidulans</i>	8	1.12	2	0.13	F
<i>A. ochraceous</i>	2	0.28	1	0.06	R
<i>A. tamarii</i>	4	0.56	2	0.13	O
<i>A. terreus</i>	8	1.12	469	30.75	C
<i>Aspergillus sp.1 (white)</i>	2	0.28	--	--	R
<i>Aspergillus sp. 2 (green)</i>	5	0.70	--	--	R
<i>Cladosporium cladosporioides</i>	2	0.28	2	0.13	R
<i>C. oxysporum</i>	1	0.14	15	0.98	F
<i>Curvularia clavata</i>	1	0.14	5	0.32	O
<i>C. eragrostidis</i>	--	--	5	0.32	R
<i>C. lunata</i>	2	0.28	17	1.11	F
<i>C. ovoidea</i>	--	--	2	0.13	R
<i>C. oryzae</i>	--	--	1	0.14	R
<i>C. robusta</i>	--	--	1	0.06	R
<i>C. tuberculata</i>	--	--	5	0.32	R
<i>Cochlioides sativus</i>	1	0.14	8	0.52	O
<i>Drechslera hawaiiensis</i>	4	0.56	1	0.06	O
<i>Fusarium lateritium</i>	7	0.98	25	1.64	C
<i>F. oxysporum</i>	13	1.83	45	2.95	C
<i>F. solani</i>	10	1.41	36	2.36	C
<i>Humicola grisea</i>	2	0.28	--	--	R
<i>Myrothecium sp</i>	1	0.14	3	0.19	O
<i>Nigrospora sphaerica</i>	--	--	1	0.06	R
<i>Paecilomyces fumorosum</i>	2	0.28	5	0.32	O
<i>P. variotii</i>	2	0.28	37	2.43	C

Table 3. Contd

<i>Penicillium citrinum</i>	4	0.56	3	0.19	O
<i>P. variabile</i>	1	0.14	2	0.13	R
<i>P. brevicompactum</i>	6	0.84	4	0.26	O
<i>P. chrysogenum</i>	--	--	2	0.13	R
<i>P. cyaneum</i>	5	0.70	--	--	O
<i>P. digitatum</i>	2	0.28	--	--	R
<i>P. expansum</i>	1	0.14	4	0.26	O
<i>P. frequentans</i>	--	--	1	0.06	R
<i>P. funiculosum</i>	--	--	1	0.06	R
<i>P. herquei</i>	17	2.40	6	0.39	F
<i>P. islandicum</i>	2	0.28	--	--	R
<i>P. ochraceum</i>	--	--	2	0.13	R
<i>P. oxalicum</i>	1	0.14	--	--	R
<i>P. purpurogenum</i>	8	1.12	5	0.32	C
<i>P. restrictum</i>	--	--	2	0.13	R
<i>P. rubrum</i>	1	0.14	9	0.59	O
<i>P. simplicissimum</i>	27	3.81	12	0.78	C
<i>P. veruculosum</i>	--	--	1	0.06	R
<i>P. waksmani</i>	--	--	11	0.72	O
<i>Penicillium sp. 6</i>	--	--	1	0.06	R
<i>Penicillium sp. MDU</i>	128	18.07	313	20.52	C
<i>Trichoderma aeroviride</i>	46	6.49	62	4.06	C
<i>T. harzianum</i>	--	--	3	0.19	R
<i>T. koningii</i>	3	0.42	--	--	R
<i>T. viride</i>	4	0.56	21	1.37	C
<i>Trichoderma sp.4</i>	2	0.28	9	0.59	O
<i>Trichurus sp</i>	--	--	4	0.26	R
<i>Unidentified sp.</i>	--	--	2	0.13	R
<b>Coelomycetes</b>					
<i>Lasiodiplodia sp.</i>	7	0.98	40	2.62	C
<i>Pestalotiopsis sp.</i>	3	0.42	10	0.65	C
<i>Phoma sp.</i>	2	0.28	6	0.39	O
<i>Phomopsis sp.</i>	--	--	20	1.31	F
<i>Nonsporulating</i>	--	--	38	2.49	

C- common; F- frequent; O- occasional; R- rare.

## Conclusions

The results obtained clearly indicate that there was a marked decrease in the number of colonies with decreasing soil micro and macro nutrients. All the macro and micro nutrients are gradually increase in the (North East resting monsoon) summer rain, nitrogen phosphorous and potassium content were rich in the second season (North East resting monsoon).

The highest number of fungal colonies was isolated from second season and micro nutrients such as; Iron and Manganese were also higher but in the case of Copper and Zinc was decreased in the second season of montane wet temperate forest. Population of soil fungi

was affected by climate. Natural moisture limitation during summer drought can constitute a stress for microbial communities in soil. It could be considered that forest soil nutrient depletion lead to a reduction and possibly the elimination of soil fungi. Seventy six taxa were isolated from montane wet temperate forest of Tamil Nadu, *Penicillium* and *Aspergillus* were dominated in both seasons due to high sporulation capacity and the *Penicillium* spp were produced fungal and bacterial antibiotics and the *Aspergillus* produced some kind of toxins such as aflatoxins, achrotoxins it may prevent the growth of other fungal species. Here there was no significant difference in their abundance and fungal distribution of both seasons, but in the case of pathogenic species,

*Fusarium laterium* was highest percent contribution and followed by *F. oxysporum* in the second season. However, most of the *Curvularia* species were represented only after summer rain.

## ACKNOWLEDGEMENT

The authors are thankful to the Prof. R. Rengasamy, Director, Centre for Advanced Studies in Botany, University of Madras, Chennai, for providing necessary laboratory facilities.

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